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# Evaluation of Platelet Reactivity in Patients With Valvular Heart Disease

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**Transmission electron microscopy with a standardized in vitro method was used to evaluate the degree of blood platelet reactivity in 72 normal subjects and 72 patients with valvular heart disease. Among the patients with abnormal natural heart valves, 51 had either aortic insufficiency or aortic stenosis, and 21 patients showed either mitral insufficiency or mitral stenosis. For normal subjects, the platelet differential counts were dominated by the dendritic type platelet, and only a few platelets showed cytoplasmic spreading between adjacent pseudopodia (spread type). A hyperactive response was defined as greater than 20% of the spread type platelet or more than 93 aggregates per 100 single platelets counted, or both. Only 6 (8%) of the 72 normal subjects showed hyperactive platelets. In contrast, 45 (62%) of the 72 patients with valvular heart disease had hyperactive platelets ($p < 0.01$). For patients with abnormal valves, the mean percent of the spread type platelet was 35% with a mean value of 105 platelet aggregates. The increased level of platelet reactivity was independent of both the position of the valve (aortic versus mitral) and its functional status (insufficient versus stenotic). Disturbed flow and the exposure of subendothelial thrombus-producing materials are features associated with abnormal heart valves. These factors, which usually occur in combination, may explain the hyperactive platelet response found in these patients.**

Several investigations (1–6) have suggested that the reactivity of blood platelets is abnormally increased in patients with valvular heart disease. A hyperactive platelet response could explain some of the pathologic features seen in these patients, such as the deposition of platelets and the formation of thrombi on the surface of altered, natural heart valves (1–4). Enhanced platelet reactivity could also contribute to some thromboembolic events known to accompany mitral valve disease (5,6). Altered platelets, if present, could then contribute to the complications of valvular heart disease, as well as participate in the development of the disease process itself. These possibilities suggested that assessing a different aspect of platelet function would broaden our understanding of the platelet response in patients with valvular heart disease.

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## Methods

**Patient data.** We evaluated platelets in 72 normal subjects and 72 patients with abnormal heart valves. In the normal group, 40 were women and 32 were men; they ranged in age from 20 to 70 years. Among the patients with valvular heart disease, 27 were women and 45 were men; they ranged in age from 14 to 81 years. The study was approved by the Henry Ford Hospital project research and Human Rights Committee on August 14, 1976. Twenty-five patients had predominant aortic insufficiency, 26 had aortic stenosis, 7 had mitral insufficiency and 14 had mitral stenosis. Only three of the patients had a history of a thromboembolic event, and all three were patients with mitral valve disease and atrial fibrillation. Two patients had episodes of cerebral ischemia, presumably due to a cerebral embolism. One patient had a pulmonary embolism. No patient with valvular heart disease was receiving platelet inhibitory drugs when platelet reactivity was studied. Specifically, patients were excluded if they received any of the following medications within 1 month of the study: aspirin, heparin, dipyridamole, allopurinol, sulfinpyrazone and propranolol.

**Platelet electron microscopic survey.** At the time we surveyed the reactivity of each platelet population using a



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standardized in vitro method (7,8), the number of circulating platelets/mm$^3$ was also determined. Three distinct types of platelets were observed when the platelets were classified at the magnification level of the transmission electron microscope. The round type was compact, had a smooth contour, was uniformly electron-dense and corresponded to the disc-shaped circulating platelet. The dendritic type was characterized by a compact, electron-dense central area from which pseudopodia extruded. The spread type showed varying degrees of cytoplasmic spreading between adjacent pseudopodia in addition to relocation of the internal organelles. A platelet differential count included the percent of round, dendritic and spread type platelets found on examination of 100 consecutive single platelets. We also recorded the number of platelet aggregates counted during the classification of the 100 single platelets.

**Statistical analysis.** We compared the percent of the spread type platelet, the number of platelet aggregates and the number of circulating platelets in the normal subjects and patients with valvular heart disease. The same variables for subgroups of patients relative to age, sex, valve position (aortic versus mitral) and functional status of the altered heart valve (insufficiency versus stenosis) were analyzed. We used the two sample *t* statistical test to determine significance. Results for the percent of the spread type platelet and number of aggregates are reported as the mean value ± 2 standard deviations.

## Results

**Normal subjects.** The number of circulating platelets in our normal subjects averaged 288,000/mm$^3$ (range 158,000 to 418,000). The differential platelet counts were consistently dominated by the dendritic type platelet (Fig. 1). The combined percent of round and dendritic types had a mean value of 92% (range 70 to 100), whereas the mean percent of the spread type was 8% (range 0 to 20). The aggregating tendency of platelet populations from normal subjects varied, with an average of 46 platelet aggregates observed per 100 single platelets counted (range 0 to 93). The ranges reflect ±2 standard deviations. Hyperactive platelet populations (more than 20 of the spread type platelet or more than 93 platelet aggregates per 100 single platelets counted, or both) were found in only 6 (8%) of the 72 normal subjects.

**Patients with valvular heart disease.** The number of circulating platelets in the patients with valvular heart disease averaged 283,900/mm$^3$ (range 127,000 to 608,000). A hyperactive platelet response was defined as greater than 20% of the spread type platelet or more than 93 aggregates per 100 single platelets counted, or both. In contrast to our normal series, in which only 8% of the subjects had a hyperactive response, 45 (62%) of the 72 patients with valvular heart disease showed a hyperactive platelet response (probability [p] $< 0.01$). The average percent of the spread type platelet (Fig. 2) was 35, with a mean of 105 platelet aggregates.

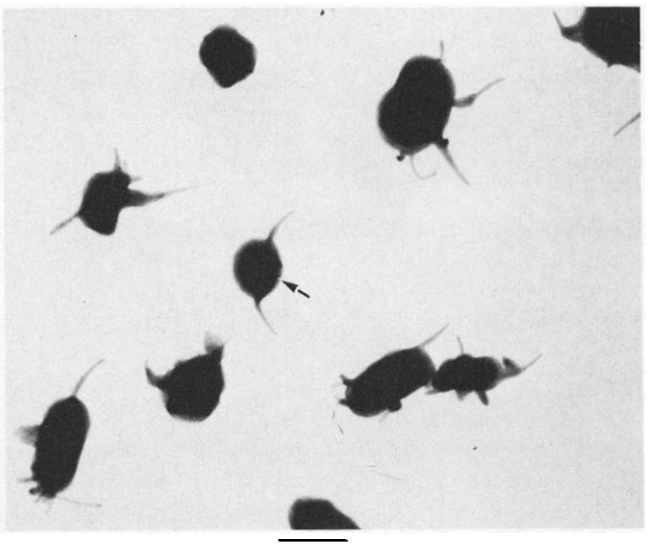
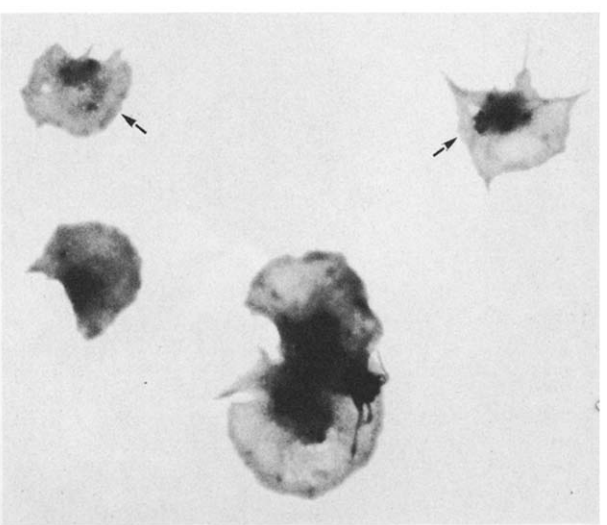


**Figure 1.** In vitro platelet response of a normal subject showing a predominance of the dendritic type platelet (**arrow**). 5,400X, reduced by 42%.

*The level of platelet reactivity appeared to be similar for various ages in our group of patients.* We determined a linear correlation between the platelet hyperactive response and age and found that the slope of the best fit curve was not statistically different from zero. Likewise, we found no significant difference in the level of platelet reactivity between female and male patients ($p > 0.05$).

*When the degree of platelet reactivity was assessed with respect to the position of the valve*, hyperactive platelet populations were found in 30 (59%) of the 51 patients with

**Figure 2.** In vitro platelet response of a patient with aortic valve disease. The spread type platelet (**arrows**) and large aggregates dominated the differential count. 4,500X, reduced by 42%.



an abnormal aortic valve and 15 (71%) of the 21 patients with mitral valve disease. The average percents of the spread type platelet were 37 and 32%, and the mean number of aggregates observed was 99 and 119, respectively (Table 1). The mean platelet count was consistently within the normal range.

*If the functional status of the diseased aortic and mitral valves was considered relevant to whether the valves were either insufficient or stenotic,* the following percents of patients within the different subgroups showed hyperactive populations of platelets: aortic insufficiency, 60% (15 of 25); aortic stenosis, 58% (15 of 26); mitral insufficiency, 100% (7 of 7) and mitral stenosis, 57% (8 of 14) (Table 1). The corresponding mean percents for the spread type platelet were 35, 38, 36 and 30%, and the associated numbers of aggregates observed were 101, 97, 146 and 106.

*No statistically significant differences ($p > 0.05$) in platelet reactivity were found for*: 1) aortic valvular heart disease versus mitral valvular heart disease; 2) aortic insufficiency versus aortic stenosis; 3) mitral insufficiency versus mitral stenosis; 4) aortic insufficiency versus mitral insufficiency; and 5) aortic stenosis versus mitral stenosis.

## Discussion

**Previous studies.** Previous reports (1–4) suggest that an altered natural heart valve may serve as a localized nidus for the thrombotic process in vivo. In a previous study (3), we used scanning electron microscopy to examine the exterior of stenotic aortic valves. We found that endothelial cells were both morphologically altered and actually lost from the surface of these valves so that subendothelial components, such as collagen, were available to the circulating blood. We further observed both single platelets and platelet aggregates adhering to exposed collagen fibers. Comparable pathologic findings (9) and Riddle et al. (unpublished data) have also been reported for the diseased, natural mitral valve, and recently, slices from abnormal mitral valves were shown to exhibit an increased thromboplastic activity combined with a decreased fibrinolytic capacity (10).

Alterations in the consumption of platelets (11) and their concentration of releasable materials (12) have also been observed in patients with valvular heart disease. Platelet survival in vivo was shortened with mitral valve disease, although it was reportedly normal in patients with aortic stenosis (11). A decreased amount of both adenosine diphosphate (ADP) and adenosine triphosphate (ATP) was released in vitro when platelet populations from patients with valvular heart disease were compared with those of normal subjects (12). This study implies that these substances were secreted from the platelets in vivo.

**Criteria of platelet reactivity.** In our study, we used a technical approach (7,8) that differed from the usual method of determining platelet reactivity in patients with valvular heart disease. Our standardized in vitro test system provided a morphologic assessment for three variables of the platelet response: adhesion, surface activation and aggregation. The presence of numerous platelets on the surface of the Formvar film reflected their adhesive capacity. Surface activation was demonstrated by the extrusion of pseudopodia, cytoplasmic spreading between pseudopodia and the reorganization of internal organelles. Aggregation, indicative of the cohesive capacity of each population of platelets, was directly observed by counting the number of platelet aggregates seen during the classification of 100 single platelets. The major advantage of this method, as opposed to the more commonly used method of aggregometry, is that one can directly observe structural changes when platelets contact an activating surface (Formvar film) in vitro.

**Effect of valvular disease on platelet reactivity.** Our study provides further evidence that the functional characteristics of platelets from patients with valvular heart disease differ from those of normal subjects. We found that more than 50% of the patients with valvular heart disease had a hyperactive platelet response; however, the degree of increased platelet reactivity did not differ significantly between aortic and mitral valve disease. Platelets were not only hyperactive as determined by our in vitro survey, but they also appeared to be more reactive in vivo as shown by our previous scanning electron microscopic observations

**Table 1.** Platelet Reactivity in Patients With Aortic or Mitral Valve Disease

| Patient Group | Patients (no.) | Patients With Hyperactive Platelets | | Average % of Spread Type Platelets | Average No. of Platelet Aggregates | Platelet Count per $mm^3$ |
|---|---|---|---|---|---|---|
| | | no. | % | | | |
| Aortic valve disease | | | | | | |
| Insufficiency | 25 | 15 | 60 | 35 | 101 | 313,000 |
| Stenosis | 26 | 15 | 58 | 38 | 97 | 248,000 |
| Total aortic valve disease | 51 | 30 | 59 | 37 | 99 | 280,000 |
| Mitral valve disease | | | | | | |
| Insufficiency | 7 | 7 | 100 | 36 | 146 | 288,000 |
| Stenosis | 14 | 8 | 57 | 30 | 106 | 295,000 |
| Total mitral valve disease | 21 | 15 | 71 | 32 | 119 | 293,000 |

(3). In both instances, adherent platelets showed dendritic processes, cytoplasmic spreading between pseudopodia and aggregate formation.

**Role of turbulent flow.** In addition to the interaction between platelets and exposed subendothelial components, the deposition of microthrombi on stenotic valves may also relate to abnormal conditions of flow. Hemodynamic factors that have been implicated in thrombus formation include high shear stresses in combination with wall interactions (13–14), turbulence (15–17) and the added effects of vortices (18).

When turbulent flow was measured in patients in the region of stenotic and regurgitant aortic valves, it was present even for mild stenosis. The intensity of turbulence was considerably greater in the jet distal to severely stenotic valves (19). Bicuspid valves, even though not significantly stenotic, may not open freely (20), causing narrowing or irregularity of the orifice which contributes to turbulent flow. Because shear stresses in turbulent jets are large as a result of additional Reynolds stresses (21), cells are likely to be damaged. The effects of shear stresses on platelet function have been studied (22–27). With various levels of stresses and different exposure times, either the structure, function or release of active substances from platelets was observed.

Platelets, leukocytes and erythrocytes may contact the arterial surface of the aortic valve by: normal backflow, valvular insufficiency (which may be associated with stenosis) and captured anular eddies. The increased adhesiveness of the platelets that we observed in patients with valvular heart disease would tend to enhance their deposition on the valve surface.

**Conclusion.** Progressive valvular disease results in an altered valve surface with which circulating peripheral blood elements including platelets, leukocytes and erythrocytes interact. Subsequent thrombus formation, thickening, fusion and calcification of the valve cusps produce disturbed patterns of flow. The hyperactive platelet response observed in our patients with valvular heart disease, therefore, probably has more than one causative factor.

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